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ORIGINAL ARTICLE

Crown architecture and leaf anatomic traits influencing herbivory on *Clethra scabra* Pers.: comparing adaptation to wetlands and drained habitats

Jaqueline Alves Pereira[1] · **Vinícius Londe**[2] · **Sérvio Pontes Ribeiro**[1] · **Hildeberto Caldas de Sousa**[1]

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Abstract We tested the hypothesis that tree species adapted to ecotones between forest-swamps have leaf structures varying in response to land water saturation (drained and wetland), which could further influence their resistance against insect herbivory. The hypothesis was tested at the crown and leaf scale. We assessed differences in the plant architecture (height, circumference at breast height, trunk height, and crown) and leaf traits (sclerophylly and anatomy) of tree species inhabiting wetland and drained soils and investigated which variables explained variance in leaf herbivory rates. The study was conducted in Montane and wetland Forests in the Itacolomi State Park, in Southern Brazil, focusing on the *Clethra scabra* Pers., which inhabits both habitats, and also *Myrcia amazonica* DC. and *Myrcia laruotteana* Cambess (congeneric) which inhabit Montane forest and wetland forest, respectively. It was found that *C. scabra* individuals in wetland areas had simpler crown structure and higher leaf sclerophylly rates than those individuals of this species in the drained habitat. However, the two *C. scabra* populations did not differ regarding overall foliage thickness and damage by herbivory between drained and wetland habitats. Variables that were related to herbivory in this species were adaxial epidermis thickness and circumference at breast height. *Myrcia* individuals from wetland areas had less damaged leaves but thinner epidermis than in drained soils, but no variable correlated to herbivory at the leaf scale. Our hypothesis was partially corroborated and suggests that *C. scabra* is more influenced by soil saturation than *Myrcia*, and its herbivory is related with structural and anatomic parameters.

Keywords Contrasting environments · Myrcia amazonica · Myrcia laruotteana · Waterlogging

Introduction

Organisms can express variable phenotypes in response to environmental conditions, the so-called phenotypic plasticity (Agrawal 2001). For plants, especially long-lived trees, plasticity is particularly important due to their sessile form of life, which requires them to deal with long-term changes in light/soil competitive conditions, plus natural enemies (Schlichting 1986). Plasticity at crown and leaf scales has been investigated for many years (Bradshaw 1965), and experiments involving plant growth in contrasting environmental conditions (for example, under sun and shade, in dry and wet habitats) have shown that populations differ remarkably regarding the same features, such as height and number of stems, and flowering time (Sultan et al. 1998; Sultan 2000).

Soil waterlogging is considered as a major abiotic stress for plants, especially because it eliminates soil air spaces, limiting gas exchanges with the atmosphere, thereby creating a hypoxic or anoxic environment (Crawford and Braendle 1996). Thus, species need adaptive strategies that enabled their survival in waterlogged soil, such as, changes in metabolic pathways in order to reduce energy

✉ Jaqueline Alves Pereira
jqalper@gmail.com

[1] Graduate Program in Ecology of Tropical Biomes, Department of Biodiversity, Evolution and Environment, Federal University of Ouro Preto, Campus Morro do Cruzeiro, s/n, Ouro Preto, Minas Gerais 35400-000, Brazil

[2] Graduate Program in Ecology, Department of Plant Biology, State University of Campinas, Av. Bertrand Russel, s/n, Cidade Universitária Zeferino Vaz, Campinas, São Paulo 13083-865, Brazil

consumption, activation of anaerobic routes, as well as changes in their morphological and anatomical structure which promote internal aeration of the plant (Davanso-Fabro et al. 1998; Medri et al. 2002).

However, certain adaptations to stressful environments can result in secondary defensive effects for the plant. For instance, plants which evolved on soils rich in heavy and/or toxic metals accumulate some of those elements in their leaves as a defense mechanism, which also end up reducing the proportion of damage caused by insect herbivores (Kazemi-Dinan et al. 2014; Plaza et al. 2015; Ribeiro et al. 2016). Little has been done to understand how much of waterlogging soil could have similar positive consequences in well-adapted plants. According to Coley et al. (1985) and Herms and Mattson (1992), it is expected that species which occupy habitats with limited resources have retarded growth and high levels of protection, since it is less costly to protect tissues from damage than to replace damaged leaves. Thus, there is a possibility that species adapted to waterlogged soils may invest in some sort of secondary defense against herbivory at a greater expense than on similar well-drained soils.

This study was based on the hypothesis that tree species adapted to ecotones between forest and swamps have leaf structures varying in response to water saturation condition of the land they occupy (drained and wetland), which could further influence their resistance against insect herbivory. The prediction is that species occupying wetland soils may be under waterlogging stress, resulting in smaller crowns, but facing harder physiological conditions, thus less capable to transporting nutrients to the leaves. Therefore, these plants should be more sclerophyllous and with thicker tissues than those of the same species or genus occupying drained soil. The more sclerophyllous the leaf is, the less palatable it is, and thus wetland plants would be less damaged by insect herbivores.

Materials and methods

Study sites–The study sites are in the Itacolomi State Park (hereafter PEIT), located in the municipalities of Ouro Preto and Mariana, Minas Gerais, Brazil. PEIT is located in the southern part of the Man and Biosphere Reserve of *Serra do Espinhaço* in the southeast of the Ferriferous Quadrangle, with a total area of 7.543 ha and an altitude ranging from 660 to 1.760 m. The climate is typically tropical with two distinct seasons (dry and wet), with a predominance of mild summers of warm days and very cold nights (Semad 2007). The region is characterized by a transition zone between two major phytophysiognomies: the Atlantic Forest and the “Cerrado” (Brazilian Savannah), both considered priority areas for conservation (biodiversity hotspots) for being rich in endemic species, alpha diversity, and with great habitat loss (Myers et al. 2000). The vegetation of PEIT is mainly composed of montane forests, montane savanna (rupestrian grasslands), and monodominant formations of *Eremanthus* spp. (Asteraceae) (Fujaco et al. 2010).

For this study, two adjacent sampling areas located on the shores of *Manso* Stream were selected, within a larger 100 ha of Montane forest, at an average altitude of 1.350 m (Pedreira and Sousa 2011): one on the right bank of the stream, covered by typical Montane forest, with sloped topography and drained soil; and another on the left bank, covered by wetland open forest, with flat topography and floodable soil (Pedreira and Sousa 2011). These two sites will be called, respectively, as drained and wetland areas (Fig. 1). Tests carried out with 37 piezometers installed in these sites have proven the water flooding or draining characteristics of these soils (Fig. 2).

Studied species– (1) *Clethra scabra* Pers. (Clethraceae) is a tree species that preferentially occurs in secondary forests, situated on slightly sloping, poor, wet, and compacted soils (Lorenzi 1998). In Brazil, it is predominantly found in areas of high altitude in the south and southeastern regions, as well as in the Amazon (Souza and Lorenzi 2005). It is a deciduous species that loses its leaves during the dry season and renews them in the rainy season, especially in the months of highest rainfall (November and December) (Pereira 2015). In PEIT, *C. scabra* occurs in the slopes of the montane forest, as well as in the adjacent wetland forest (Pedreira and Sousa 2011).

(2) *Myrcia amazonica* DC. (Myrtaceae) is an evergreen tree species that renews its leaves during the dry season. It has broad geographic distribution in Brazil, occurring from the State of Amazonas to Santa Catarina, typical of the savanna riparian forests, semi-deciduous seasonal forests, and montane savannas (Sobral 2007). In the PEIT, *M. amazonica* is only found in the montane forest (drained area).

(3) *Myrcia laruotteana* Cambess. (Myrtaceae) is an evergreen tree species that also renew its leaves during the dry season. It occurs in Argentina, Paraguay, and Brazil (Sobral et al. 2013). This species is very frequent in semi-deciduous forests, especially in riparian zones and wetland areas. In PEIT, *M. laruotteana* only occurs in the wetland forest (wetland area).

Sampling design and data collection–Within each study area, four transects of 10 m each were inserted as a grid for the identification of targeted species. In the drained area, all lines were installed parallel to the *Manso* stream, and in the wetland area, three lines were allocated parallel to the stream and one perpendicular, to select locations with well-defined soil characteristics from each site. All individuals from the three species in an area of 10 m^2 around the lines,

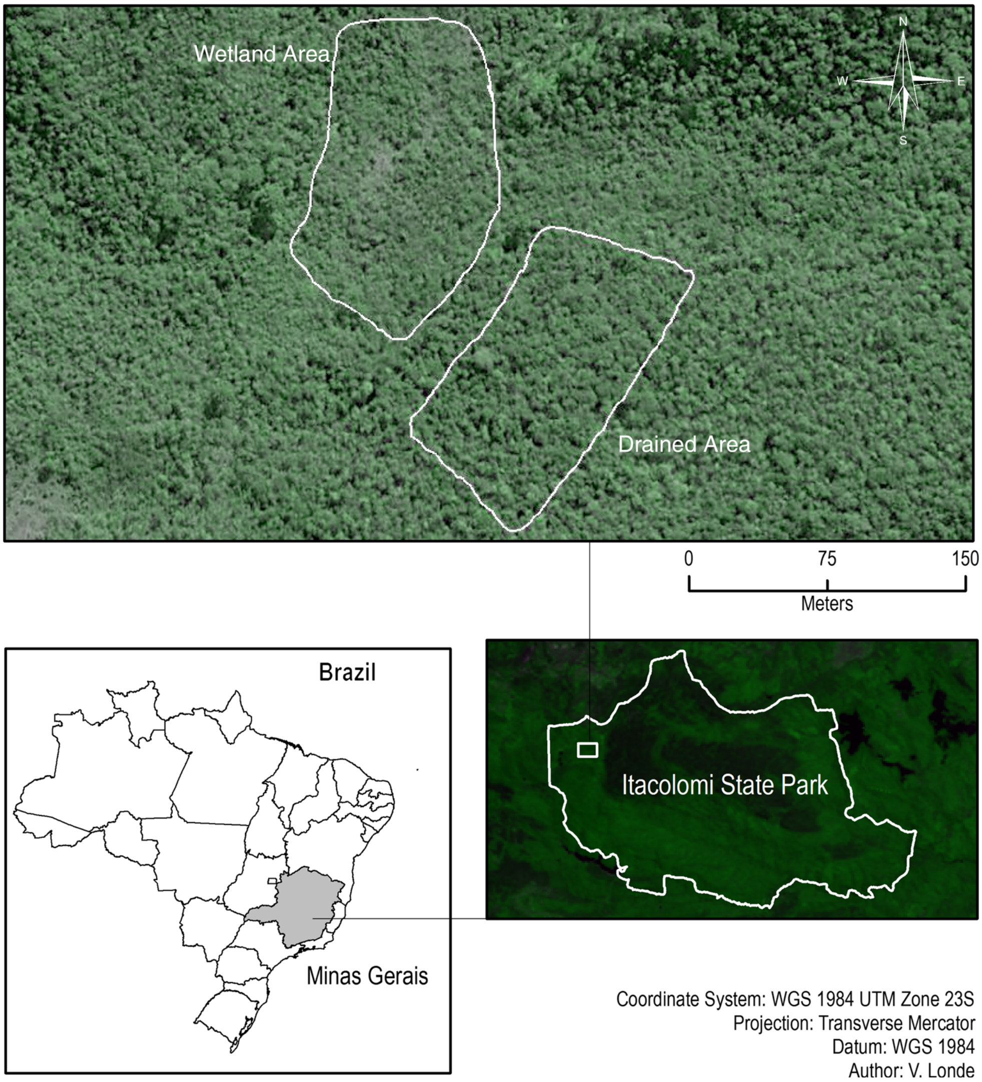


Fig. 1 Location of the studied areas in the Montane and Wetland Forest at Itacolomi State Park, Minas Gerais State, southeast Brazil. Coordinates: 20°25′38.53″S; 43°30′19.03″W

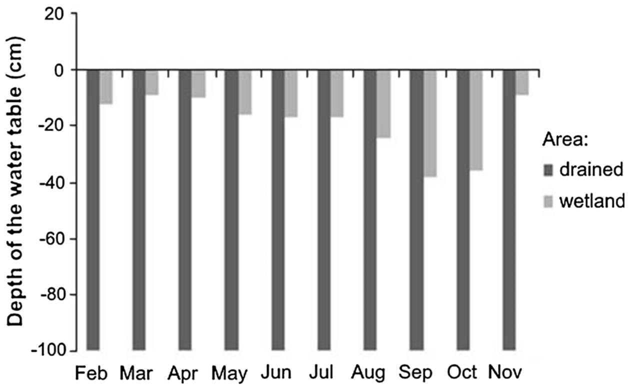


Fig. 2 Water table surface depth measurements with piezometers, proving that wetland areas remained constantly waterlogged throughout the data collection period, and that the water did not reach the surface of the soil (>1 m) in the drained areas. Data collected in 2014 in Montane and Wetland Forest in Itacolomi State Park, Minas Gerais

with circumference at breast height (CBH) equal to or greater than 15 cm, were identified and labeled with numbered stickers. Then, within the set of numbered individuals, 20 trees of each species were randomly sampled by sorting pieces of paper with the correspondent number of the individuals, summing up to 80 plants (20 *C. scabra* + 20 *M. amazonica* from drained areas / 20 *C. scabra* + 20 *M. laruotteana* from wetland areas). Overall height, trunk height, crown depth—the difference between total height and trunk height—and CBH of each individual were taken.

Herbivory–In order to verify the ratio of damaged leaves by insect chewing herbivores for each species, samples of botanical material were collected in late April and early May 2014, a period that coincides with the end of the rainy season, when most of leaf damage already happened, and then are accumulated in already old leaves (Ribeiro et al.

1994; Marquis et al. 2002). Access to the tree canopy was performed by free climbing techniques with ropes and also with ladder assistance. Three branches from independent branching sides were sampled per plant, and from each branch the total number of leaves and the proportion of damaged leaves by chewing herbivores were quantified.

For estimating the rate of herbivory damage, we used the leaf area per class estimation method, following the methodology proposed by Dirzo and Domínguez (1995). Fifty leaves from each branch (150 leaves/plant) were selected at random, and leaf area lost was estimated within the following classes: 0 to 5%, 6 to 25%, 26 to 50%, 51 to 75%, 76 to 95%, and 100% damage. Then, the following formula was used to calculate Relative Average of Damaged Leaves (RADL) per plant:

$$\mathrm{RADL} = \sum \frac{(\text{number of leaves referent to damage class per branch x class median})}{\text{number of leaves per branch}}.$$

This method is the most parsimonious if one takes large classes of damage, based on the median damage within each class. Hence, it is essential to better characterize herbivory in large tree crowns, as damage can vary dramatically among branches (Ribeiro et al. 1994; Ribeiro and Brown 1999). Besides, it is a well-accepted and largely used method in insect–plant literature.

Sclerophylly–To ascertain whether the leaves of some species were more sclerophyllous, two mature and herbivory-free leaves were selected from each branch (6 leaves/plant) to determine its leaf specific mass (LSM = dry weight/leaf area) used to estimate leaf sclerophylly (Turner 1994). The selected leaves were scanned and the total leaf area was measured using Sigma Scan software. To determine the dry weights of leaves, they were dried in an oven at 60 °C for 72 h and then weighed on an analytical scale.

Leaf anatomy–In the field, six plants from each species were selected in each habitat in late April and early May 2014; two leaves per branch (6 leaves/plant—different from those used to estimate sclerophylly) were sampled from each individual. Leaves were numbered, stored in plastic bags, packed in Styrofoam boxes with ice, and sent to the Plant Anatomy Laboratory of the Federal University of Ouro Preto. At the laboratory, the leaves were fixed in FAA_{70} for 48 h and then preserved in 70% alcohol (Johansen 1940).

Next, histological cross sections were performed freehand using razor blade and marrow's petiole of *embaúba* (*Cecropia* sp.) as support. The sections were stained with astra blue and basic fuchsine, following the protocol proposed by Kraus and Arduin (1997), and arranged on glass slides with 50% glycerol. Besides that, were performed histochemical tests of fresh material which were freehand sectioned and subjected to reaction with ferric chloride and phloroglucinol to visualize, respectively, phenolic compounds and lignified or suberous cell walls (Johansen 1940; Kraus and Arduin 1997). An overall description of leaf anatomy of each species was made, and the thicknesses of anatomical structures such as of abaxial and adaxial epidermis, palisade and spongy parenchyma, mesophyll and limb were measured with the aid of a digital camera (5.0-megapixel resolution) coupled to a Zeiss optical microscope using AxionVision 4.8 software.

Data analysis–To test whether the crown architecture (structural parameters) varied between individuals of the same species or between those in the congeneric ones, comparing the two habitats, Student *t* tests were performed (at 95% confidence interval), and to verify significant differences in anatomical variables, one-way ANOVA was performed. To test the prediction that stress from waterlogged soil influences the structure of the plant crown, an analysis of covariance (ANCOVA) was performed by analyzing the effect of the total height, trunk height, crown depth, and CBH on mean leaf area lost by herbivory. To test whether the condition of waterlogged soil would influence the sclerophylly and consequently leaf herbivory, an analysis of variance (one-way ANOVA) was performed checking differences in sclerophylly between species and the types of habitat they occupied. To ascertain differences in leaf herbivory of species among environments, a two-way ANOVA was performed.

As some leaf anatomical structures could be collinear, a pre-selection of least correlated explanatory variables via collinear analyses and stepwise multiple linear regression was performed. The anatomical characteristics of lacunar parenchyma, palisade parenchyma, mesophyll, and leaf blade had high collinearity, while the abaxial and adaxial epidermis showed no redundancy and were used in subsequent analyses. Finally, an optimized model that could identify which parameters (structural, anatomical, or sclerophylly) could better explain herbivory rates through stepwise multiple regressions was performed (Zuur et al.

2007). All statistical analyses were carried out using the free software R 3.2.0 (R Core Team 2015).

Results

Qualitative anatomical description of leaves–The leaves of *C. scabra* are predominantly dorsiventral, but sometimes they may have isobilateral areas, and hypostomatic with thin cuticle in both faces (Fig. 3). The epidermis of the adaxial face is multi-stratified with two or three cell layers, while the abaxial face has one layer (Fig. 4). The palisade parenchyma has one or two cell layers and the lacunar parenchyma is lax with two to four layers (Fig. 4). The abaxial epidermis is covered by many stellate trichomes (Fig. 5). The vascular bundles have one sclerenchymatous sheath extending to the epidermis of both faces, with thin-walled fibers filled by phenolic substances (Fig. 5).

The leaves of *Myrcia amazonica* is glabrous, dorsiventral, and hypostomatic with one cell layer; the cell walls and cuticles are thin in both faces (Fig. 6). The palisade parenchyma has two cell layers, and the lacunar is lax with eight to ten layers (Fig. 6). Secretory glands are found in the palisade parenchyma and midrib (Fig. 6). The epidermal cells of both faces, the parenchymal cells of the mesophyll, and those of the midrib, and have phenolic compounds (Figs. 7, 8). The vascular bundles have a sclerenchymatous sheath with one to three cell layers (Fig. 9).

Myrcia laruotteana also has glabrous leaves, and they are dorsiventral and hypostomatic with one cell layer in both faces. The cuticle is thin, and the palisade parenchyma is peculiar with two cell layers (Fig. 10). The lacunar parenchyma is lax with six to eight cell layers. Secretory glands are present in the palisade and lacunar parenchyma (Fig. 10), and the vascular bundles have a sheath with sclerenchymatous fibers (Fig. 11).

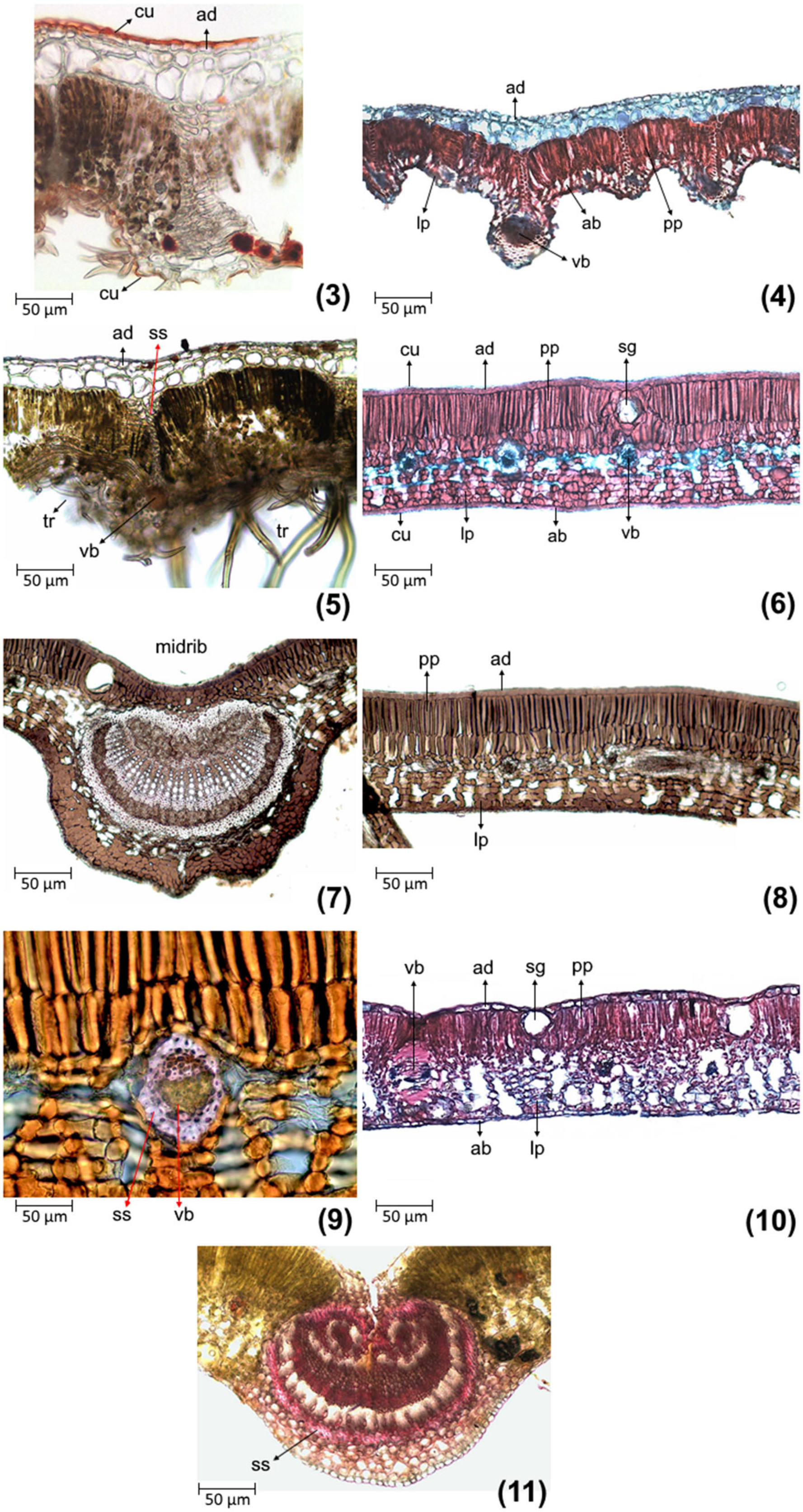
Tree structure, sclerophylly, quantitative anatomy, and herbivory–All structural tree crown parameters in *C. scabra* pointed towards smaller crowns in wetland than in drained area (Table 1). On the contrary, mean leaf sclerophylly was significantly higher in *C. scabra* plants in the wetlands than in the drained habitat ($F_{(238)} = 19.46$; $P < 0.001$) (Fig. 12). Individuals of *C. scabra* in the wetland area had thicker anatomic structures than the drained ones—except for abaxial epidermis (Table 2). However, there was no significant difference in the epidermis thicknesses between areas ($F_{(70)} = 0.371$; $P = 0.54$ adaxial epidermis, and $F_{(70)} = 0.01$; $P = 0.91$ abaxial epidermis). The proportion of leaf damage in *C. scabra* was 19% in the wetland area and 23% in the drained area, with a marginally significant difference between them ($F_{(38)} = 3.40$; $P = 0.07$) (Fig. 13).

For *Myrcia*, the mean height of trees and the trunk height were lower in the wetland area than in the drained one (Table 1). The leaves of the plants inhabiting the drained soils were more sclerophyllous; however, they did not differ significantly from the wetland soil plants ($F_{(238)} = 1.57$; $P = 0.21$). In the drained habitat, plants of *M. amazonica* had all anatomic structures significantly thicker than individuals of *M. laruotteana* in the wetland area (Table 2). The proportion of leaf damage in *Myrcia* was 5.5% in the wetland area and 9% in the drained one, with a significant difference between them ($F_{(38)} = 13.95$; $P < 0.001$) (Fig. 13). Comparing all plants between soil types, it was found that plants from both species in wetland soils had less leaf area lost than plants that inhabited drained soils ($F_{(78)} = 11.55$; $P = 0.001$), and that *Myrcia* individuals suffered less damage than *C. scabra* ($F_{(78)} = 187.42$; $P = 0.000$) (Fig. 13).

Leaf herbivory was only negatively related with the adaxial epidermis thickness, among all the tested anatomical parameters in *C. scabra*, regardless of habitat. This means that increased thickness of adaxial epidermis leads to a decrease in leaf damage ($r^2 = 62.5$) (Fig. 14a). Among the structural parameters, it was found that *C. scabra* individuals in the drained areas with greater CBH had more leaf damage, while in wetland areas individuals with lower CBH had with higher leaf damage ($R^2 = 57.68$) (Fig. 14b). In the model selection, these two factors (thickness of adaxial epidermis and CBH) together better explained the leaf herbivory in *C. scabra* ($R^2 = 81$). In *Myrcia* species, leaf herbivory did not vary in response to anatomic traits, and nor did the plant architecture ($r^2 = 5$ and 7, respectively).

Discussion

Since the 80s, ecologists have been trying to understand which are the mechanical defenses that are most effective in refraining insect herbivory (Strong et al. 1984), and how these and chemical traits vary among habitat conditions (Gershenzon 1984; Coley et al. 1985). Mechanical defense is an evolutionary long-term process, and amazing physical and physiological adaptive responses have appeared in specialized insect, then, those that can keep eating and massing up any pattern (Herms and Mattson 1992; Marquis et al. 2002). Still, here we found that increasing adaxial epidermis thickness reduces herbivory in *C. scabra*. More than just overall sclerophylly, the location and physical structure of a sclerophyllous trait seem to be an effective defense, a similar one to the well-studied trichome density (Ribeiro et al. 1994; Valverde et al. 2001; Molina-Montenegro et al. 2006). Plants that invest in a thicker epidermis, perhaps by compensating the thin cuticle, provide



Figs. 3–11 Leaf anatomy of *Clethra scabra* (**3**–**5**), *Myrcia amazonica* (**6–8**), and *Myrcia laruotteana* (**9–11**). These species were studied in drained and wetland areas at the Itacolomi State Park, Ouro Preto, Minas Gerais. Fig. **3** was stained with Sudan III; Figs. **4**, **6**, and **10** were stained with astra blue and fuchsine; Figs. **5**, **7**, and **8** were reacted with ferric chloride—*dark color* (*black* and *brown*) indicates a strong reaction and the presence of phenolic compounds-; and Figs. **9** and **11** were reacted with phloroglucinol—*pink color* indicates the presence of lignin. Abbreviations: *ad* adaxial epidermis, *ab* abaxial epidermis, *cu* cuticle, *vb* vascular bundles, *pp* palisade parenchyma, *lp* lacunar parenchyma, *tr* trichomes, *ss* sclerenchymatous sheath, *sg* secretory glands. To have complete description of leaf anatomy, please, see the text. (Color figure online)

Table 1 Structural data (mean) of tree species studied in the drained and wetland areas at the Itacolomi State Park, southeastern Brazil

	Clethra			*Myrcia*	
	Drained	Wetland		Drained	Wetland
CBH (cm)*	50.55	20.68	CBH (cm)	25.03	32.03
Height (m)*	9	5.25	Height (m)	8.33	7
Trunk (m)	5.93	3.58	Trunk (m)*	6.25	1.31
Crown (m)	3.07	1.67	Crown (m)*	2.08	6.94

* Significant difference in the parameter ($P < 0.05$), in the same species or genera, between areas

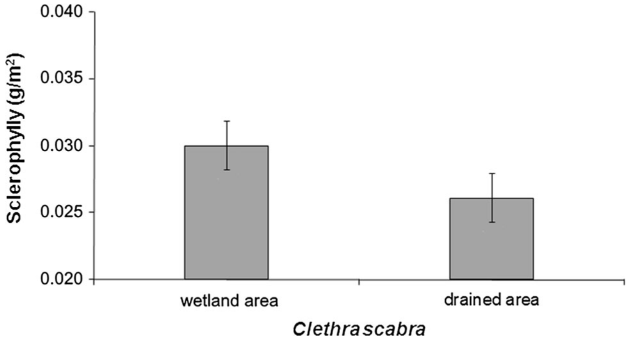


Fig. 12 Leaf sclerophylly of *Clethra scabra* differed significantly among both study areas in the Itacolomi State Park, Minas Gerais, with plants from the wetland habitats being more sclerophyllous. *Vertical bars* represent standard error

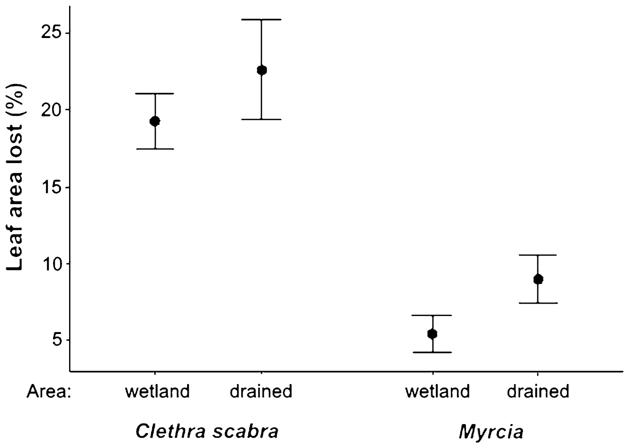


Fig. 13 Leaf herbivory was higher in *Clethra scabra* individuals, mainly from the drained areas, but there was no significant difference between areas. For *Myrcia*, although leaves were less consumed by herbivores, the percentage of leaf area lost was significantly higher in plants from the drained areas. Data collected in Montane and Wetland Forests in Itacolomi State Park, Minas Gerais. Vertical bars represent standard error

their insect herbivores an extra barrier that deters their process of predation, by avoiding access to deeper tissues. Of course, this is not an isolated defensive trait, confounding with *C. scabra's* numerous trichomes, fibers around the vascular bundles and phenolic substances filling the sclerenchymatous sheath.

However, interestingly, even with this set of defense mechanisms, the predation on *C. scabra* was higher and suggests that other factors, perhaps those related to population structure (tree density, for example), may be influencing its predation. On the other hand, *Myrcia* species were less damaged, despite they had leaves less provided with physical defenses (the lack of trichomes, e.g.), evidencing that a more obvious accumulation of secondary chemicals is probably, such as phenolic compounds, more densely detected in the tissues of *M. amazonica* than others.

From the set of structural parameters, CBH was an important variable related to herbivory in *C. scabra*, but in different measures. One possible explanation for the fact that plants with higher CBH in the drained areas were more damaged by herbivorous insects is that maybe these plants are older, higher, and probably more apparent to herbivores (hypothesis of the appearance of plants—Feeny 1976). *Clethra scabra* plants in the drained areas would therefore be diluted in a canopy, where species with higher and larger crowns could attract more herbivores (Lawton 1983; Fleck and Fonseca 2007; Neves et al. 2013). On the other hand, in the wetland area, there was a relation between reduced CBH and increased herbivory, probably due the abundance of individuals of *C. scabra* with smaller crown

Table 2 Mean of leaf anatomy parameters assessed in the tree species of the drained and wetland areas at the Itacolomi State Park, southeastern Brazil. All measures are in micrometer (µm)

	Clethra			*Myrcia*	
	Drained	Wetland		Drained	Wetland
Adaxial epidermis	46.97	48.48	Adaxial epidermis*	15.30	11.73
Abaxial epidermis	12.25	12.22	Abaxial epidermis*	12.28	9.94
Palisade parenchyma*	81.16	85.85	Palisade parenchyma*	157.16	107.41
Lacunar parenchyma*	37.94	42.13	Lacunar parenchyma*	160.27	112.24
Mesophyll*	117.39	124.13	Mesophyll*	308	217.57
Leaf blade*	173.04	184.74	Leaf blade*	343.24	242.41

* Significant difference in the parameter ($P < 0.05$), in the same species or genera, between areas

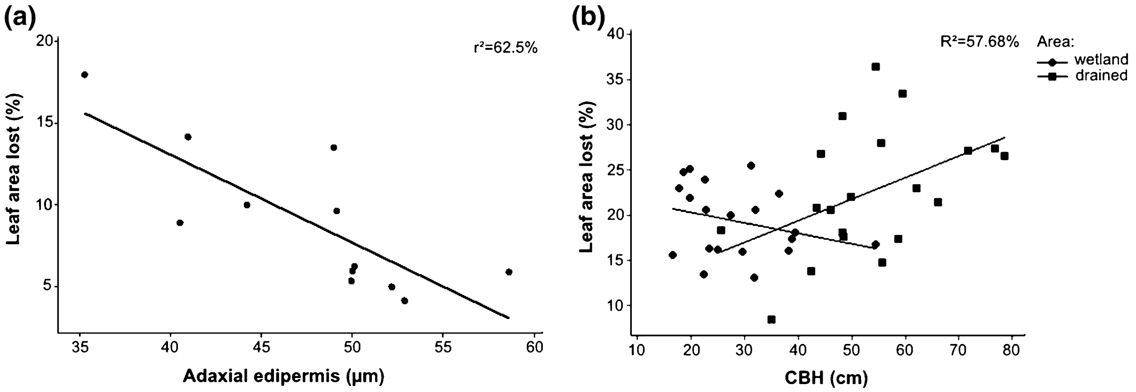


Fig. 14 Loss of leaf area in *Clethra scabra* was correlated to adaxial epidermis thickness (**a**) and to circumference at breast height (CBH) (**b**), in the wetland and drained areas of Itacolomi State Park, Minas Gerais

in this area (Pedreira and Sousa 2011), and perhaps because they are more susceptible to herbivores.

However, considering all the studied species, as predicted, the plants from the wetland area were less herbivored than plants from the drained area, although the variables have explained herbivory from just one of the studied species and without distinction between habitats. Such result suggests that factors other than those evaluated may be influencing the herbivory on plants of the wetland area. It is possible, for example, that plants of wetland soils would face a constant trade-off: investing in growth and development, or maintenance of waterlogging stress, and this may lead to a production of a less amount of leaves and so less herbivory. It is known that some processes occur in soils with water saturation that directly affect the plants that inhabit them. For example, various reduction reactions decrease the oxygen concentration which, in turn, causes the decrease of essential elements is the vegetation, such as nitrates, manganese, iron, sulfates, and carbon dioxide (Hurt 2005). Thus, plants tend to grow and develop less (Larcher 2000), as observed in CBH, height, trunk, and crown for *C. scabra*, and in height and trunk for *M. laruotteana*, probably affecting the production of leaves too. Other factors may be the presence and abundance of insect predators, whether they are specialists or generalists, and also the structural factors such as density of individual trees that can facilitate or hinder the chances of the herbivore finding plants (Espírito-Santo et al. 2012).

Regarding the prediction that plants found in wetland soils would be more sclerophyllous than those in drained soils, it was only partially confirmed, as only *C. scabra* showed this pattern. However, direct sclerophylly measure did not explain herbivory in none of the species. This outcome suggests that sclerophylly may not be a defense strategy but an adaptation to stressful conditions, probably to excessive solar radiation (Jordan et al. 2005), and perhaps to low-nutrient soil at the wetland area (Loveless 1961), but with less consequences for insects, maybe due to their adaptation to such conditions.

Also, the prediction that species from the wetland area would form smaller canopies but thicker anatomical tissues, with a consequent reduction of leaf area lost, was partially confirmed. As discussed above, the waterlogging may be influencing the trees' structure; however, comparing the structural and anatomic data (Tables 1, 2), it can be noticed that the stress by excessive solar radiation also may have an important role on the dynamics of trees; because while *C. scabra* had smaller crown at the wetland habitat but thicker chlorophyllian parenchyma (palisade + lacunar parenchyma), in *Myrcia* the opposite occurred, i.e., with the reduction of the crown at the drained habitat, but with the same effect of leaves exposed to sunlight, with thicker chlorenchyma.

On contrary to *Myrcia* species, *C. scabra* leaf area lost by herbivores was high in comparison to that described in the literature. In tropical forests, the average annual rates of leaf damage are between 7 and 11.1% (Coley and Barone 1996; Metcalfe et al. 2014), and in the savannah and dry forests the average annual rates of leaf damage are 18 and 14%, respectively (Ribeiro 2003), and *C. scabra* had a mean of 21% leaf area lost. The reason for the high rates of herbivory in tropical forest regions lies in the fact that these regions shelter a high diversity of plant species which provide different niches that can be occupied by a range of herbivorous insects in the same habitat (MacArthur 1967; Coley and Barone 1996). As discussed above, specialization results in a coevolutionary war race between defenses and damage, which must result in high herbivory where insect diversity is high. Still, the variation in damage between habitats and individuals shows that herbivory selective pressure is variable, and may keep the host plant under constant change in its adaptation to soil versus herbivores.

Furthermore, recent studies have increasingly shown the importance of top-down control on insect herbivores. Thus, predators and parasites cannot be ignored as a key aspect in herbivory distribution. Canopy habitats can result in a very diversified scenario where insect herbivores may prefer worse food under safer conditions than otherwise (Ribeiro et al. 2005; Ribeiro and Basset 2007, 2016). The widespread presence of territorial ant species in Itacolomi forest was proved to affect insect herbivore distribution among tree crowns and forest physiognomies (Lourenço et al. 2015). Unfortunately, there are limited data available on the life history of tropical tree species to produce a broader, theoretical sound conclusion. Leaf anatomy became a neglected aspect of biology, and this study tries to bring about data and patterns to contribute to a change in our understanding of tropical insect–tree interaction.

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